Abstract

In this thesis chromatographic conditions for the HPLC analysis of ribonucleotides AMP, ADP and ATP were optimized. For their separation chromatographic column, Nucleogel SAX 1000-8, 50 x 4,6 mm, from the German company Marcherey-Nagel was used. The mobile phase of a 0,2 mol/L solution of KH_2PO_4, its pH was adjusted with 1mol/L with potassium hydroxide to pH = 5,00. As the most suitable flow rate was selected 1,0 mL/min. The analysis was conducted using an isocratic elution. Detection was conducted by a DAD detector at a wavelength of $\lambda = 260$ nm. Substances were eluted in the following order AMP ($t_r = 0{,}911$ min), ADP ($t_r = 1{,}667$ min) and ATP ($t_r = 7{,}262$ min). The total analysis time of the mixture of standards, under the conditions mentioned above, lasted for 10 minutes.

For the extraction of adenosine-5-ribonucloetides the real matrix frozen and lyophilized leaves of tobacco virginia (*Nicotiana tabacum L.*) have been used. Two methods of extraction were applied. The first procedure was based on the extraction of AMP, ADP and ATP from the leaves of the tobacco plant by boiling deionized water. In the second method 0,07 mol/L $HClO_4$ for the extraction instead of deionized water was used.

Keywords: HPLC, adenosine ribonucleotides, AMP, ADP, ATP, tobacco virginia